P/1259-503

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Fernand LABRIE

Serial No.: 09/405,678 Group Art Unit: 1617

Filed: September 24, 1999 Examiner: S. Wang

For: MEDICAL USES OF A SELECTIVE ESTROGEN RECEPTOR MODULATOR IN COMBINATION WITH SEX STEROID PRECURSORS

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF FERNAND LABRIE UNDER 37 C.F.R. §1.132

Sir:

I, Fernand Labrie, having a residence at 2989 de la Promenade, Sainte-foy (Quebec) G1W 2J5, Canada, hereby declare that:

1. I am an inventor of the above-identified application and am familiar with the subject application.

2. I received a Ph.D. in Endocrinology from Laval University in 1966. I am presently Director of the Laboratory of Molecular Endocrinology at Le Centre de Recherche du Centre Hospitalier de L'Universite Laval (CHUL) in Quebec City, Canada, and am also Director of the Department of Physiology at Laval University and Scientific Director of Le Centre de Recherche du Centre Hospitalier de L'Universite Laval.

3. The following study was undertaken under my supervision to study the effect on the levels of serum cholesterol in ovariectomized female rats following 3 months of treatment with EM-652 HCl (i.e., EM-1538), administered alone or in combination with dehydroepiandrosterone (DHEA).

4. Ten to twelve week-old female Sprague-Dawley rats (Crl:CD(SD)Br) (Charles River Laboratory, St. Constant, Canada) weighing approximately 220-270g at start of treatment were

used. The animals were acclimatized to the environmental conditions (temperature: 22 ± 3°C; humidity: 50 ± 20%; 12-h light-12-h dark cycles, lights on at 07:15h) for at least 1 week before starting the experiments. The animals were housed individually and were allowed free access to tap water and a pelleted certified rodent feed (Lab Diet 5002, Ralston Purina, St. Louis, MO). Experiments were conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) in accordance with the CCAC Guide for Care and Use of Experimental Animals.

5. Eighty-four rats were randomly distributed between 6 groups of 14 animals each as follows: 1) Intact control; 2) OVX control; 3) OVX + E_2 (1 mg/kg); 4) OVX + EM-652.HCl (2.5 mg/kg); 5) OVX + dehydroepiandrosterone (DHEA; 80 mg/kg); 6) OVX + DHEA + EM-652.HCl. One day 1 of the study, the animals of the appropriate groups were bilaterally ovariectomized (OVX) under isoflurane anesthesia. The DHEA was applied topically on the dorsal skin as a solution in 50% ethanol-50% propylene glycol while the other tested compounds were administered as suspension in 0.4% methylcellulose by oral gavage. Treatments were initiated on day 2 of the study and were performed once daily for 3 months.

6. After 3 months of treatment, blood samples were collected at the jugular vein from overnight fasted animals (under Isoflurane anesthesia). Samples were processed for serum preparation and frozen at -80°C until assay. Serum cholesterol levels were determined using the Boehringer Mannheim Diagnostic Hitachi 911 Analyzer (Boehringer Mannheim Diagnostic Laboratory Systems).

7. Data are expressed as means ± SEM. Statistical significance was determined according to the multiple-range test of Duncan-Kramer (Kramer CY; Biometrics 1956, 12:307-310).

8. As shown in the Table below, three months after ovariectomy, a 22% increase in serum cholesterol levels was observed in OVX control rats compared to intact controls ($P<0.01$). In fact, serum cholesterol was increased from 2.01 ± 0.11 mmol/L in intact animals to 2.46 ± 0.08 mmol/L in OVX controls. The administration of E_2 or DHEA alone decreased serum cholesterol levels to 1.37 ± 0.18 mmol/L and 1.59 ± 0.10 mmol/L, respectively, while the

administration of EM-652.HCl alone or in combination with DHEA led to cholesterol levels significantly lower (0.87 ± 0.04 and 0.65 mmol/L, respectively) than those found in intact animals (2.01 ± 0.11 mmol/L). Moreover, cholesterol levels reached with the combination are significantly lower than those obtained with DHEA ($p<0.01$) or EM-652.HCl ($p<0.05$) alone. Again, since DHEA can form androgens and/or estrogens, such an effect of the combination therapy cannot be predicted.

Table

EFFECT ON SERUM CHOLESTEROL LEVELS FOLLOWING 3 MONTH TREATMENT WITH ESTRADIOL, EM-652.HCl OR DHEA, ADMINISTERED ALONE OR IN COMBINATION, TO OVARIECTOMIZED FEMALE RATS

Treatment	Cholesterol (mmol/L)
Intact	2.01 ± 0.11**
OVX	2.46 ± 0.08
OVX + E_2	1.37 ± 0.18**
OVX + EM-652.HCl	0.87 ± 0.04**†
OVX + DHEA	1.59 ± 0.10**††
OVX + DHEA + EM-652.HCl	0.65 ± 0.06**

** $p<0.01$, experimental versus OVX control rats.
† $p<0.05$, EM-652.HCl-treated rats versus EM-652.HCl+DHEA-treated rats
†† $p<0.01$, DHEA-treated rats versus EM-652.HCl+DHEA-treated rats.

9. I further declare that all statements made herein are made of my own knowledge and are true except for those statements made on information and belief, which are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this declaration of this application and any United States patent issuing therefrom.

June 26, 2001
Date

Fernand Labrie